

Molecular screening of carbapenemase Encoding genes in *Pseudomonas aeruginosa* from Tertiary Healthcare in South Eastern Nigeria.

ABSTRACT

Background and Objectives

In recent years, the rate of carbapenemase encoding gene in *P. aeruginosa* has increased worldwide and has become of great concern since it's significantly restricts the therapeutic options for patients in Tertiary health care. Therefore, there's a need for Molecular screening of carbapenemase encoding genes in *Pseudomonas aeruginosa* from Tertiary Healthcare in South Eastern Nigeria.

Methodology

A total of twelve (12) *Pseudomonas aeruginosa* positive culture of Urine (n=5), Wound swab (n=5), Catheter tip (n=2) were collected from Alex Ekwueme Federal University Hospital Teaching Hospital, Abakaliki (AE-FUTHA), Ebonyi State, South eastern Nigeria. The *Pseudomonas aeruginosa* strain confirmation was performed using VITEK 2 System and the bacteria were further screen for carbapenemase encoding gene by PCR specific primer.

Result:

Molecular amplification of carbapenemase encoding genes revealed that *bla*NDM and *bla*IPM accounted 12 (100%) across all sample source. Among the various sample sources, *bla*KPC was found 1(8.3%) in Urine, wound swab 3(25.0%), and Catheter tip 1(8.3%), while *bla*VIM was found 2(16.7%), 2(16.7%) and 0(0.0%) in Urine, wound swab and Catheter tip respectively. Co-expression of *bla*NDM + *bla*IMP accounted 5(41.6 %), 5(41.6 %) and 2(16.7 %) in Urine, wound swab and Catheter tip respectively. Co-expression of *bla*KPC + *bla*NDM + *bla*VIM + *bla*IMP + *bla*OXA was only detected in urine 1(8.3 %).

Conclusion

The current study gives an account of the presence of carbapenemase-encoding genes in *P. aeruginosa*. The expression of carbapenemase-encoding genes may be the mainstay of phenotypic MDR. As a result, physicians, other medical professionals, researchers, and public health policymakers must be kept up to date on the spread of carbapenemase-encoding genes. In addition, strict infection prevention and control strategies, as well as antimicrobial stewardship programs, are highly desirable in admission healthcare facilities where carbapenemase-encoding genes are spreading.

Keyword: Carbapenemase-encoding, Gene, *Pseudomonas aeruginosa*

1. INTRODUCTION

Pseudomonas aeruginosa is an opportunistic and nosocomial pathogen that colonizes people with cystic fibrosis and is a common etiologic agent of bacteremia, urinary tract infections, wound infections, and colonizes people with cystic fibrosis, among other infections. Recently, it has been described as a pathogen that co-infects patients with COVID-19 [1, 2]. Despite the advances made through the introduction of anti-pseudomonal drugs, *Pseudomonas aeruginosa* infections remain a big threat in patients due to its resistance to antibiotics. Most drugs have lost their efficacy against *Pseudomonas aeruginosa* and the susceptibility patterns change with time and regions in the world. The increase in antibiotic resistance by *Pseudomonas aeruginosa* has been observed in several epidemiological studies carried out all over the world [3].

Currently the major threat of antibiotic-resistant bacteria is from MDR Gram-negative organisms, particularly those which have developed resistance to carbapenem. Along with carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), are among the top tier of the WHO list of antibiotic-resistant "priority pathogens" that pose the greatest threat to human health [4, 5]. Among the clinically important bacteria that harbor plasmidic genes encoding a wide variety of carbapenemases, isolates of sequence type (ST) 258, ST11, and ST147 Enterobacterales (mainly

producing *Klebsiella pneumoniae* carbapenemase [KPC], metallo- β -lactamase [MBL, especially New Delhi MBL {NDM}], and oxacillinase [OXA], etc.), ST111 and ST235 *Pseudomonas aeruginosa* (mainly producing NDM, imipenemase [IMP], Verona integron encoded MBL [VIM], and OXA, etc.) [6]. *P. aeruginosa* isolates have been reported to contain a wide variety of carbapenemases encoding gene globally. For example, in Latin America, this includes KPC, IMP, VIM, NDM [7]. In the Arabian Peninsula, carbapenemases in *P. aeruginosa* include VIM, IMP, and NDM [7]. In the United States, carbapenemases in *P. aeruginosa* include KPC, NDM, VIM, and IMP [8, 9]. In another study, the pooled prevalence of among the clinical specimens in Africa OXA and VIM were the most prevailing carbapenemase among *P. aeruginosa* [10]. In recent years, the rate of carbapenemase encoding gene in *P. aeruginosa* has increased worldwide and has become of great concern since it significantly restricts the therapeutic options for patients in health care [11]. Notably, increase in carbapenemase encoding gene in clinically important *P. aeruginosa* has gradually worsened in hospitalized in patients [6, 12]. The risk of colonization with carbapenemase encoding *P. aeruginosa* isolates harboring blaVIM, blaKPC, blaIMP, blaNDM was also reported to apparently increase with the length of hospital stay [7, 13]. The emergence and rapid spread of carbapenemase encoding gene is a global concern as infections with these resistant bacteria are a matter of national and international concern as they are an emerging cause of Hospital Acquired Infections (HAIs) that pose a significant threat to public health and responsible for hospital outbreaks worldwide, thus, managing infections caused by them poses a substantial challenge in clinical practice. Hence a robust and feasible examination of carbapenemase encoding gene in *P. aeruginosa* will serve as a guide regarding their clinical management, including prevention and treatment.

2. MATERIALS AND METHODS

2.1 Identification of *Pseudomonas aeruginosa*

The approval for this study was conveyed with the Ethical clearance number SMOH/ERC/043/21 obtained from the Ministry of Health Ebonyi State's research and ethics committee. *Pseudomonas aeruginosa* positive culture of Urine (n=5), Wound swab (n=5) and Catheter tip (n=2) were collected from Alex Ekwueme Federal University Hospital Teaching Hospital, Abakaliki (AE-FUTHA), Ebonyi State, South eastern Nigeria. The *Pseudomonas aeruginosa* strain confirmation was performed using VITEK 2 System (bioMerieux, France) [14, 15].

2.2 Molecular Screening for Carbapenemase Encoding Genes Using Polymerase Chain Reaction (PCR)

2.2.1 Genomic DNA Extraction

Genomic DNA Extraction of *Pseudomonas aeruginosa* was performed using ZR Fungal/Bacterial DNA Miniprep™ (Manufactured by Zymo research, cat number: D6005) kit according to the manufacturer's protocol. Exactly 2 ml of bacterial cells broth was added to a ZR Bashing TM Lysis tube and 750 μ g lysis solutions was added to it. This was secured in a bead fitted with 2 ml tube holder assembly and processed at a maximum speed for 5 minutes. The ZR Bashing Bead Tm lysis tube was centrifuged at $> 10,000 \times g$ for 1 minute. Up to 400 μ g of supernatant was transferred to a Zymo-Spin TM IV Filter in a collection tube and centrifuged at $7,000 \times g$ for 1 minute. Exactly 1,200 μ g of fungal/bacterial DNA Binding Buffer was added to the filtrate in the collection tube. Exactly 800 μ l of the mixture from step 5 was transferred to a Zymo-spin™ IIC Column in a collection tube and centrifuged at $10,000 \times g$ for 1 minute. The flow through from the collection tube was discarded. Then 200 μ l DNA Pre-washed buffer was added to the Zymo-Spin TM IIC Column in a new tube collection and centrifuged at $10,000 \times g$ for 1 minute. About 200 μ g of Fungal/Bacterial DNA Buffer was added to the Zymo-Spin TM IIC Column and centrifuged at $10,000 \times g$ for 1 minute. The Zymo-Spin TM IIC Column was transferred to a clean 1.5 ml micro-centrifuge tube and 100 μ l (35 μ l minimum) DNA was added directly to the column matrix. Centrifuge was done at $10,000 \times g$ for 30 seconds to elute DNA [16].

2.2.2 Electrophoresis for DNA and PCR

Exactly 1 g of agarose was measured (for DNA) and 2 g of agarose for PCR products. Agarose powder was mixed with 100 ml 1xTAE in a microwave flask. This was microwaved for 1-3 min until agarose is completely dissolved. The agarose solution was allowed to cool to about 50 °C. Then 10 μ g EZ vision DNA stain was added. The agarose was poured into a gel tray with the well comb in place. The newly poured gel was placed at 4 °C for 10-15 mins until it has completely solidified [16].

2.2.3 Loading Samples and Running an Agarose Gel.

A loading Buffer was added to each DNA sample of PCR products. Once it got solidified the agarose gel was placed into the gel box (electrophoresis unit). The gel box was filled with 1xTAE buffer until the gel was covered. A molecular weight ladder was carefully loaded into the first lane of gel and the samples were carefully loaded into the additional wells of gel. The gel was run at 80-150 V for about 1-1.5 h. The power was turned off, and the electrodes

disconnected from the power source and then the gel was carefully removed from the gel box. The DNA fragments or PCR fragments was visualized under UV trans-illuminator [16, 17].

2.2.4 PCR Mix Components

The PCR mix components was made up of 12.5 μ l of Taq 2 x Master Mix from New England Biolabs (M0270); 1 μ l each of 10 μ m forward and reverse primer (Invitrogen, U. S. ATM) (Table 1) [18, 19]; 2 μ l of DNA template and then made up with 8.5 μ L Nuclease free water. The PCR reactions was executed as previously described [16, 17].

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Table 1 : Primer sequence for Screening of carbapenemase encoding gene

Target gene	Primer sequence (5'-3')	Size (bp)	Cycling Condition
<i>blaIMP</i>	F: GGAATAGAGTGGCTTAAYTCT R: CGGTTTAAAYAAAACAACCACC	232	Initial denaturation at 94°C x 5mins, followed by 34 cycles of denat. at 94°C x 30 secs 42°C x 30 secs (annealing) 72°C x 5 mins secs(elongation)
<i>blaVIM</i>	F: GATGGTGTGGTTCGCATA R: CGAATGCGCAGCACCAG	250	Initial denaturation at 94°C x 5mins, followed by 34 cycles of denat. at 94°C x 30 secs 47°C x 30 secs (annealing) 72°C x 5 mins secs(elongation)
<i>blaKPC</i>	F: CGTCTAGTTCTGCTTAGGCG R: CTTGTCATCCTTGTAAACG	500	94°C x 5mins, followed by 34 cycles of denat. at 94°C x 30 secs 58.8°C x 30 secs (annealing) 72°C x 5 mins secs(elongation)
<i>blaOXA</i>	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	550	94°C x 5mins, followed by 34 cycles of denat. at 94°C x 30 secs 52°C x 30 secs (annealing) 72°C x 5 mins secs(elongation)
<i>blaNDM</i>	F: GGTGGCGATCTGGTTTTTC R: CGGAATGGCTCATCACGATC	800	33 cycles at 95°C x 3mins 65°C x 30secs followed by 1 cycle at 72°C x 10mins

3. RESULT

3.1 Occurrence of Carbapenemase encoding Gene among *Pseudomonas aeruginosa* from different clinical sample.

Among different sample source, *blaKPC* was detected 1(8.3 %) from Urine, wound swab 3(25.0 %) and Catheter tip 1(8.3 %) followed by *blaVIM* found 2(16.7 %) from Urine, wound swab 2(16.7 %) and Catheter tip 0(0.0 %) while *blaNDM* and *blaIPM* accounted 12 (100%) across all sample source as shown in Table 2.

3.2 Co-expression of Carbapenemase Encoding Gene among *Pseudomonas aeruginosa* from different clinical sample.

Co-expression of *blaNDM* + *blaIMP* accounted 5(41.6 %), 5(41.6 %) and 2(16.7 %) in Urine, wound swab and Catheter tip respectively. Co-expression of *blaKPC* + *blaNDM* + *blaVIM* + *blaIMP* + *blaOXA* was only detected in urine 1(8.3 %) as shown in Table 3.

Table 2: Occurrence of Carbapenemase encoding Gene among *Pseudomonas aeruginosa* from different clinical sample

Sample source	Urine(n=5)	Wound swab (n=5)	Catheter tip(n=2)	Total (n=12)
Isolate coding	P1, P2, P3, P4, P5	P6, P7, P8, P9, P10	P11, P12	
Gene				
<i>blaKPC</i>	1(8.3 %)	3(25.0 %)	1(8.3 %)	5(41.7 %)
<i>blaNDM</i>	4(33.3 %)	4(33.3 %)	4(33.3 %)	12(100 %)
<i>blaVIM</i>	2(16.7 %)	2(16.7 %)	0(0.0 %)	4(33.3%)
<i>blaIMP</i>	4(33.3 %)	4(33.3 %)	4(33.3)	12(100%)
<i>blaOXA</i>	3(25.0 %)	1(8.3 %)	1(8.3 %)	5(41.7 %)

KEY: n - number of isolate, *blaKPC*-*Klebsiella pneumoniae* carbapenemase, *blaIMP*-Imipenemases, *blaVIM*-Verona integron Metallo-beta-lactamase, *blaNDM*-New Delhi Metallo-beta-lactamase, *blaOXA*-Oxacillinase.

Table 3: Co-expression of Carbapenemase Encoding Gene among *Pseudomonas aeruginosa* from different clinical sample

Sample source	Urine(n=5)	Wound swab(n=5)	Catheter tip(n=2)	Total (n=12)
Isolate coding	P1, P2, P3, P4, P5	P6, P7, P8, P9, P10	P11, P12	
Co-expression				
<i>bla</i> NDM + <i>bla</i> IMP	5(41.6 %)	5(41.6 %)	2(16.7 %)	12(100 %)
<i>bla</i> KPC+ <i>bla</i> NDM+ <i>bla</i> VIM+	1(8.3 %)	0(0.0 %)	0(0.0 %)	1(8.3%)
<i>bla</i> IMP+ <i>bla</i> OXA				

KEY: %-Percentage, n-Number of isolate, *bla*KPC-*Klebsiella pneumoniae* carbapenemase, *bla*IMP-Imipenemases, *bla*VIM-Verona integron Metallo-beta-lactamase, *bla*NDM-New Delhi Metallo-beta-lactamase, *bla*OXA-Oxacillinase

4. DISCUSSION

Both *blaIMP* and *blaNDM100%* was the most predominant gene identified among *P. aeruginosa* while *blaVIM* 4(33.3 %) was the least identified gene. In contrast, several studies reported *blaVIM* gene as the most common MBL found in CR- *P. aeruginosa* [20, 21] while in addition, the most prevalent carbapenemase encoding genes in *P. aeruginosa* are the VIM and IMP types; particularly, VIM-2 has become the dominant carbapenemase encoding gene type worldwide [22] but in accordance to a systemic review of carbapenase gene in *P. aeruginosa*, IMP followed by NDM have become the two most prevalent class B carbapenemases in worldwide *P. aeruginosa* isolates [11]. Also, in a previous study conducted in Iran *blaIMP* was reported as the most prevalent carbapenemase gene in *P. aeruginosa* isolates [23]. A retrospective screening of *P. aeruginosa* identified the *blaIMP* gene in 1992 in Japan [24]. Subsequent outbreaks due to the transferable drug resistance conferred by the gene were reported [25].

Although, *blaOXA* 41.7 % and *blaVIM* 33.3 % were detected. Their presence could be linked to the observed phenotypic MDR due to efflux pump and AmpC beta-lactamase overproduction. Similarly, a study conducted by Kao *et al.* [26] who analyzed the resistance mechanisms in 87 BSI-causing imipenem-resistant *P. aeruginosa* isolates collected in southern Taiwan between 2000 and 2010, revealed that carbapenemases (mainly VIM and OXA), active efflux pumps, and AmpC beta-lactamase overproduction were found in 10.3%, 74.4%, and 51.3% of the *P. aeruginosa* isolates, respectively [26]. Class D enzymes have a wide range of substrates and include OXA enzymes, which in general are not inhibited by traditional beta-lactamase inhibitors e.g., tazobactam, ticarcillin (used in this study) and may not be inhibited by new agents (e.g., vaborbactam, relebactam). OXA gene variants such as OXA-10 has been shown to exhibits ESBL activity and confers cefoxitin, ceftazidime and aztreonam resistance, while OXA- 31 confers resistance to cefepime [27, 28] which are commonly use antibiotics in healthcare for the treatment enterobacteria infection in Nigeria.

P. aeruginosa isolates producing KPC are mainly identified in Europe and Asia [11] but detection of 5(41.7 %) KPC-producer in this study reveals a continental spread and may have been acquired from other enterobacteria such as *Klebsiella pneumoniae* and *E. coli* were they are predominantly found.

The emergence of *blaNDM-1* and *blaIMP* encoding isolates at surgical outpatient ward, and *P. aeruginosa* at intensive care unit can severely compromise the safety of vulnerable patients admitted to the hospital. As noted in this study, IMP producing *P. aeruginosa* are the carbapenemase-producing strains mainly circulating at our hospital environment. The widespread *P. aeruginosa* clone is often associated with poor clinical outcomes due to its

multidrug resistance and virulence factors, representatively the cytotoxin *ExoU* causing necrotic cell death [11, 29, 30, 31]. It has also been reported that New Delhi metallo-beta-lactamase (NDM producing strains are responsible for outbreaks throughout Europe, Asia, and South America [32] and its role in Pseudomonal infection in this hospital may not be underestimated.

Co-expression of *blaKPC* + *blaNDM* + *blaVIM* + *blaIMP* + *blaOXA* was only detected in one isolate 1(8.3 %). The carbapenem resistance determinants carried by *P. aeruginosa* are often encoded on plasmids, such as IncP type; class I integrons, such as those carrying the *blaOXA*, *blaIPM*, *blaKPC*, *blaVIM* gene; and other mobile genetics elements, such as those associated with insertion sequences with a common region (ISCRs), which enhance the organism's ability to disseminate resistance among multiple species [7, 11]. In addition, these isolates frequently carry additional resistance determinants that diminish the clinical utility of the beta-lactam, fluoroquinolones, aminoglycosides etc., as observed *in vitro* in this study. Carbapenemase producing *P. aeruginosa* are often resistant to all of these therapeutic options, thus making treatment failure a likely outcome.

CONCLUSION

The current study provides baseline information on the occurrence of carbapenemase-encoding genes carried by *P. aeruginosa*. The expression of these genes could be the mainstay of phenotypic MDR. Therefore, physicians, other medical professionals, researchers, and public health policymakers must be informed about the spread of this carbapenemase encoding genes. Also, in admission healthcare facilities where carbapenemase encoding genes are spreading, strict infection prevention and control strategies, as well as antimicrobial stewardship programs, are highly desirable.

CONSENT

All authors have declared that no competing interests exist.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Qu J, Cai Z, Liu Y, Duan X, Han S, Liu J, Zhu Y, Jiang Z, Zhang Y, Zhuo C. Persistent Bacterial Coinfection of a COVID-19 Patient Caused by a Genetically Adapted *Pseudomonas aeruginosa* Chronic Colonizer. *Front Cell Infect Microbiol.* 2021 11:641920.
2. Loyola-Cruz MÁ, Durán-Manuel EM, Cruz-Cruz C, Marquez-Valdelamar LM, Bravata-Alcantara, JC, Cortés-Ortíz IA, Cureño-Díaz MA, Ibáñez-Cervantes G, Fernández-Sánchez V, Castro-Escarpulli G. ESKAPE bacteria Characterization Reveals the Presence of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* outbreaks in COVID-19/VAP patients. *Am J Infect Cont.* 2022; 20:22-67.

3. Rajat RM, Ninama GL, Mistry K, Parmar R, Patel K, Vegad MM. Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care Hospital, Ahmadabad, *National J Med Res.* 2012; 2(11):156-9.
4. Willyard C. The Drug Resistant Bacteria that Pose the Greatest Health Threats. *Nature.* 2017; 543:15-78.
5. Sheu CC, Chang YT, Lin SY, Chen YH, Hsueh PR. Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Update on Therapeutic Options. *Front Microbiol.* 2019; 10:80-89
6. Jean SS, Ko WC, Lu MC, Lee WS, Hsueh PR. Multicenter Surveillance of In Vitro Activities of Cefepime-Zidebactam, Cefepime- Enmetazobactam, Omadacycline, Eravacycline, and Comparator Antibiotics Against Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter Baumannii* Complex Causing Bloodstream Infection in Taiwan 2020. *Exp Rev Anti Infect Ther.* 2022; 1:13-45.
7. Tenover FC, Nicolau DP, Gill CM. Carbapenemase producing *Pseudomonas aeruginosa* –an Emerging Challenge. *Emerg Microbes Infect.* 2022; 11:1811-814
8. Walters MS, Grass JE, Bulens SN, Hancock EB, Phipps EC, Muleta D. Carbapenem-resistant *Pseudomonas aeruginosa* at US Emerging Infections Program Sites, 2015. *Emerg Infect Dis.* 2019; 25:1281–1288.
9. Poirel L, Nordmann P, Lagrutta E, Cleary T, Munoz-Price LS. Emergence of KPC-producing *Pseudomonas aeruginosa* in the United States. *J Antimicrob Agent Chemother.* 2010; 54:3072.
10. Kindu M, Derseh L, Gelaw B, Moges F. Carbapenemase-Producing Non-Glucose-Fermenting Gram-Negative Bacilli in Africa, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: A Systematic Review and Meta-Analysis. *Int J Microbiol.* 2020; 94:61-901.
11. Yoon EJ, Jeong SH. Mobile Carbapenemase Genes in *Pseudomonas aeruginosa*. *Front Microbiol.* 2021; 12:614-058
12. Davoudi-Monfared E, Khalili H. The Threat of Carbapenem- Resistant Gram-Negative Bacteria in a Middle East Region. *Infect Drug Resist.* 2018; 11:1831–1880.
13. Neidhöfer C, Buechler C, Neidhöfer G, Bierbaum G, Hannel I, Hoerauf A. Global Distribution Patterns of Carbapenemase-Encoding Bacteria in a New Light: Clues on a Role for Ethnicity. *Front Cell Infect Microbiol.* 2021; 11:659-753.
14. Edemekong, CI, Iroha IR, Thompson, MD, Okolo, IO., Uzoeto HO, Ngwu JN, Mohammed ID, Chukwu EB, Nwuzo AC, Okike BM, Okolie SO, Peter IU. Phenotypic Characterization and Antibigram of Non-Oral Bacteria Isolates from Patients Attending Dental Clinic at Federal College of Dental Technology and Therapy Medical Center Enugu. *Int J Pathog Res.* 2022; 11(2): 7-19.
15. Peter IU, Ngwu JN, Edemekong CI, Ugwueke IV, Uzoeto HO, Joseph OV, Mohammed ID., Mbong EO, Nomeh OL, Ikusika BA, Ubom IJ, Inyogu JC, Ntekpe ME, Obodoechi IF, NseAbasi PL, Ogbonna IP, Didiugwu CM, Akpu PO, Alagba EE, Ogba RC, Iroha IR. First Report Prevalence of Livestock Acquired Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) Strain in South Eastern, Nigeria. *IOSR J Nurs Health Sci.* 2022;11(1)-50-56.
16. Peter IU, Emelda NC, Chukwu EB, Ngwu JN, Uzoeto HO, Moneth EC Stella AO, Edemekong CI, Uzoamaka EP, Nwuzo AC, Iroha IR. Molecular Detection of Bone Sialoprotein-Binding Protein (*bbp*) Genes among Clinical Isolates of Methicillin Resistant *Staphylococcus aureus* from Hospitalized Orthopedic Wound Patients. *Asian J Orthopaedic Res.* 2022, 8(3): 1-9.
17. Edemekong CI, Uzoeto HO, Mbong EO, Ikusika BA, Didiugwu, CM, Ngwu JN, NseAbasi PL, Ntekpe ME, Mohammed ID, John-Onwe BN, Alagba EE, Obodoechi IF, Joseph OV, Ogbonna IP, Ubom IJ, Peter IU (2022). Molecular Characterization and Bioassay of Soil *Actinomycetes* Strains on Multidrug Resistant Bacteria. *IOSR J Biotechnol Biochem.* 2022; (1):6-11.
18. Aruhomukama D, Najjuka CF, Kajumbula H, Okee M, Mboowa G, Sserwadda I. blaVIM- and blaOXA-mediated Carbapenem Resistance among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* Isolates from the Mulago Hospital Intensive Care Unit in Kampala, Uganda. *BMC Infect Dis.* 2019; 19:853.
19. Verma N, Prahraj AK, Mishra B, Behera B, Gupta K. Detection of Carbapenemase-producing *Pseudomonas aeruginosa* by Phenotypic and Genotypic Methods in a Tertiary Care Hospital of East India. *J Lab Physiol.* 2019; 11:287–291.

20. Ellappan K, Belgode NH, Kumar S. Coexistence of Multidrug Resistance Mechanisms and Virulence Genes in Carbapenem-resistant *Pseudomonas aeruginosa* Strains from a Tertiary Care Hospital in South India. *J Glob Antimicrob Resist*. 2018; 12:37–43.
21. Al Dawodeyah HY, Obeidat N, Abu-Qatouseh LF, Shehabi AA. Antimicrobial Resistance and Putative Virulence Genes of *Pseudomonas aeruginosa* Isolates from Patients with Respiratory Tract Infection. *Germs*, 2018; 8(1):31–40.
22. Walsh TR. Clinically Significant Carbapenemases: An Update. *Current Opin Infect Dis*. 2008; 21(4):367–371.
23. Dogonchi, A. A., Ghaemi, E. A., Ardebili, A., Yazdansetad, S and Pournajaf, A. (2018). Metallo- β -lactamase-mediated Resistance among Clinical Carbapenem-resistant *Pseudomonas aeruginosa* isolates in Northern Iran: A Potential Threat to Clinical Therapeutics. *Ci ji yi xue za zhi and Tzu-chi Med J*. 2018; 30(2), 90–96.
24. Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka S. PCR Detection of Metallo-beta-lactamase gene (blaIMP) in Gram-negative rods Resistant to Broad-spectrum Beta-lactams. *J Clin Microbiol*. 1996a; 34:2909–2913
25. Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K. Multifocal Outbreaks of Metallo-beta-lactamase-producing *Pseudomonas aeruginosa* Resistant to Broad-spectrum beta-lactams, Including Carbapenems. *J Antimicrob Agent Chemother*. 1996b; 40:349–353.
26. Kao CY, Chen SS, Hung KH, Wu HM, Hsueh PR, Yan JJ. Overproduction of Active Efflux Pump and Variations of *OprD* Dominate in Imipenem-Resistant *Pseudomonas aeruginosa* Isolated From Patients with Bloodstream Infections in Taiwan. *BMC Microbiol*. 2016; 16:107–456.
27. Bush K, Jacoby GA. Updated Functional Classification of Beta-lactamases. *J Antimicrob Agent Chemother*. 2010; 54(3):969–76
28. Kunz Coyne AJ, Amer E, Dana H, Nicholas R, Michael J R. Therapeutic Strategies for Emerging Multidrug- Resistant *Pseudomonas aeruginosa*. *Infect Dis Ther*. 2022; 11:661–682
29. Sato H, Frank DW, Hillard CJ, Feix JB, Pankhaniya RR, Moriyama K. The Mechanism of Action of the *Pseudomonas aeruginosa*-encoded Type III Cytotoxin, ExoU. *EMBO J*. 2003; 22:2959–2969.
30. Roy-Chowdhury P, Scott MJ, Djordjevic SP. Genomic Islands 1 and 2 Carry Multiple Antibiotic Resistance Genes in *Pseudomonas aeruginosa* ST235, ST253, ST111 and ST175 and are Globally Dispersed. *J Antimicrob Agent Chemother*. 2017; 72:620–622.
31. Yoon EJ, Kim D, Lee H, Lee HS, Shin JH, Park YS. Mortality Dynamics of *Pseudomonas aeruginosa* Bloodstream Infections and the Influence of Defective *OprD* on Mortality: Prospective Observational Study. *J Antimicrob Agent Chemother*. 2019; 74:2774–2783.
32. Moran-Barrio J, Lisa MN, Larrieux N, Drusin SI, Viale AM, Moreno DM. Crystal structure of the Metallo-beta-lactamase Gob in the Periplasmic Dizinc form Reveals an Unusual Metal Site. *J Antimicrob Agent Chemother*. 2016; 60:6013–6022.